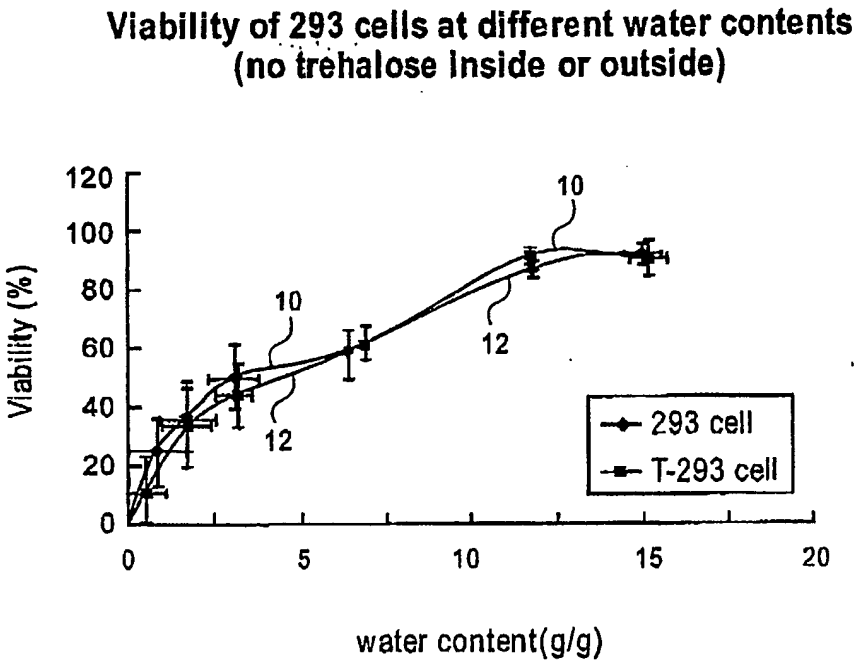

Viability of 293 cells at different water contents
(no trehalose inside or outside)
Viability (%)
120
100
80
60
40
20
0
0
5
10
15
20
water content(g/g)
10
10
12
12
293 cell
T-293 cell

FIG. 1

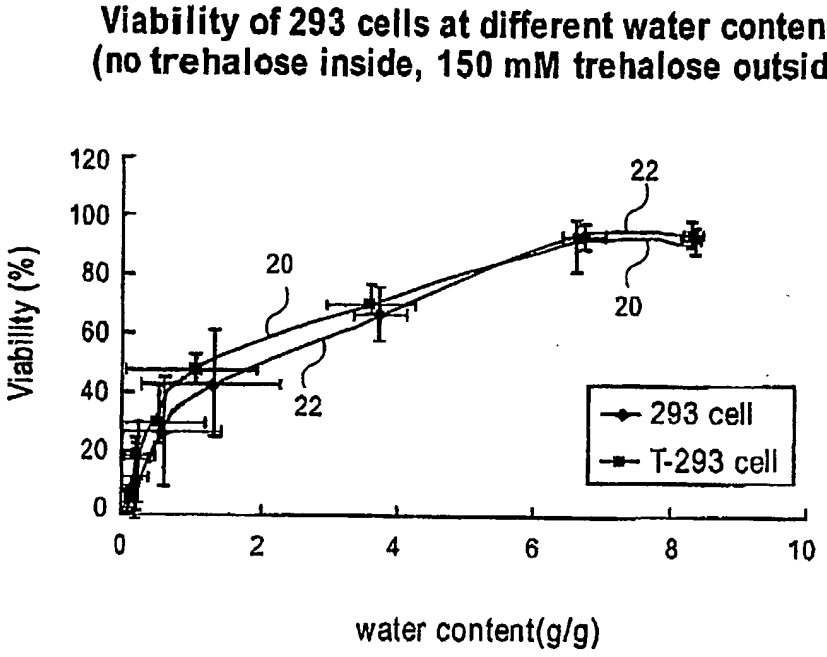

Viability of 293 cells at different water contents
(no trehalose inside, 150 mM trehalose outside)
Viability (%)
120
100
80
60
40
20
0
0
2
4
6
8
10
water content(g/g)
20
22
20
22
293 cell
T-293 cell

FIG. 2

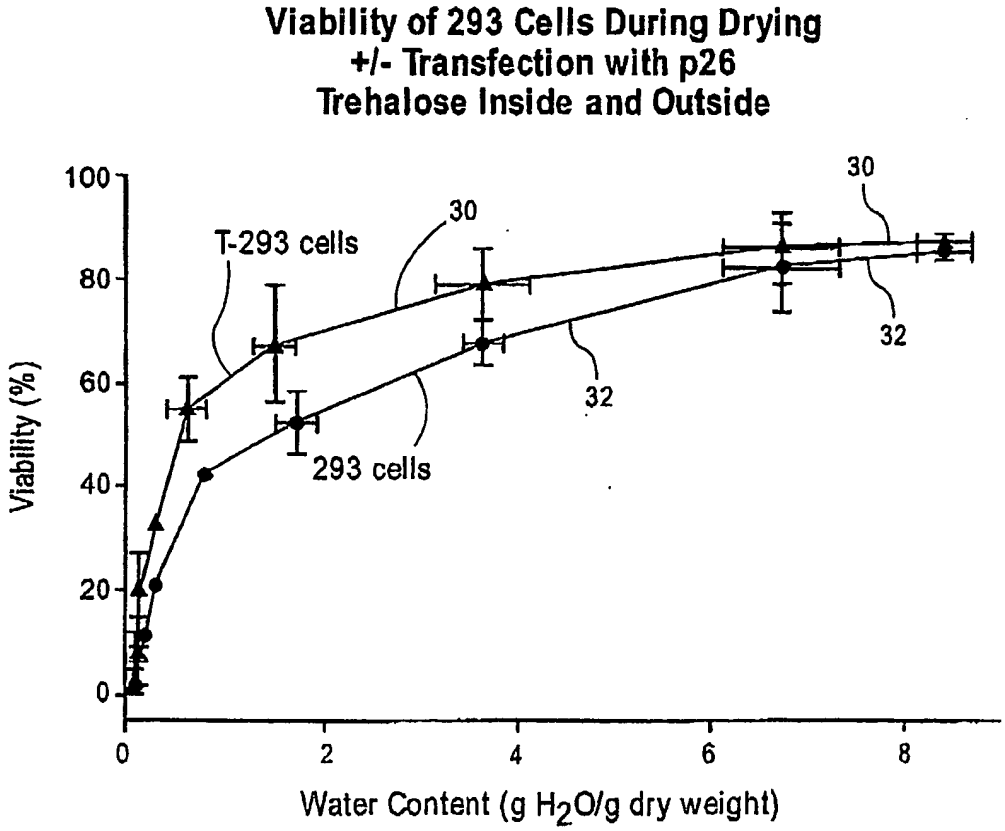

Viability of 293 Cells During Drying
+/- Transfection with p26
Trehalose Inside and Outside
Viability (%)
100
80
60
40
20
0
0
2
4
6
8
Water Content (g H2O/g dry weight)
T-293 cells
293 cells
30
32
30
32

FIG. 3

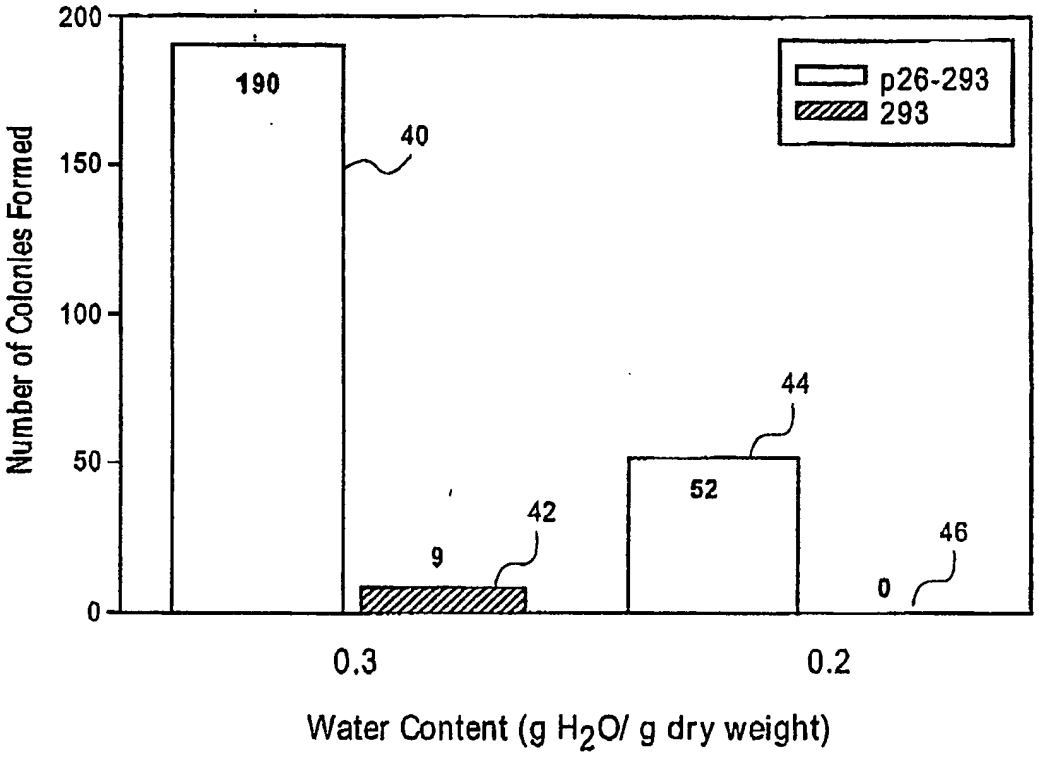

200
150
100
50
0
Number of Colonies Formed
190
40
9
42
52
44
0
46
p26-293
293
0.3
0.2
Water Content (g H_2O/ g dry weight)

FIG. 4

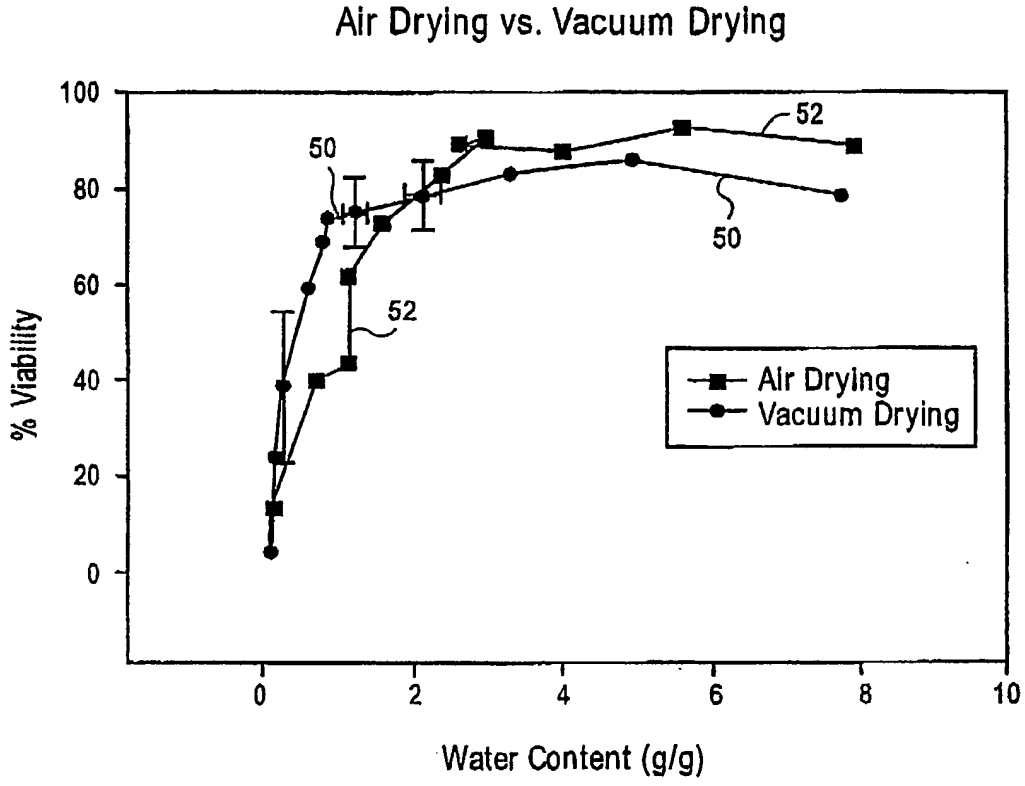

Air Drying vs. Vacuum Drying
% Viability
Water Content (g/g)
Air Drying
Vacuum Drying
50
52
50
52
0
20
40
60
80
100
0
2
4
6
8
10

FIG. 5

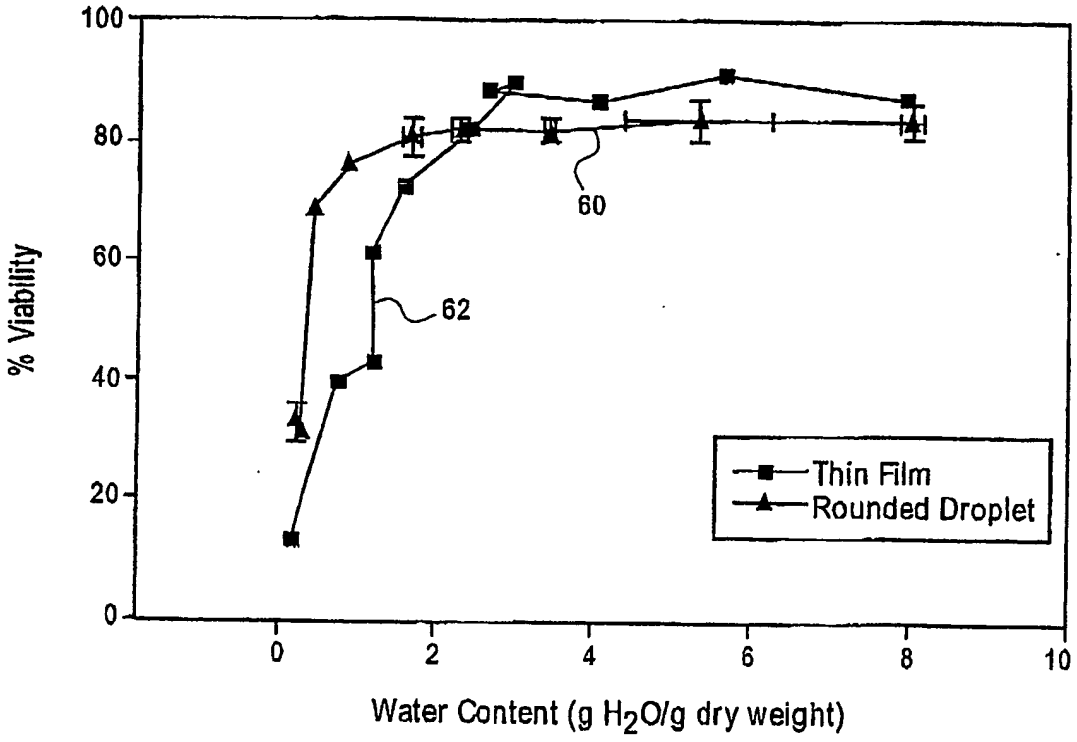

100
80
60
40
20
0
% Viability
0
2
4
6
8
10
Water Content (g H_2O/g dry weight)
60
62
Thin Film
Rounded Droplet

FIG. 6

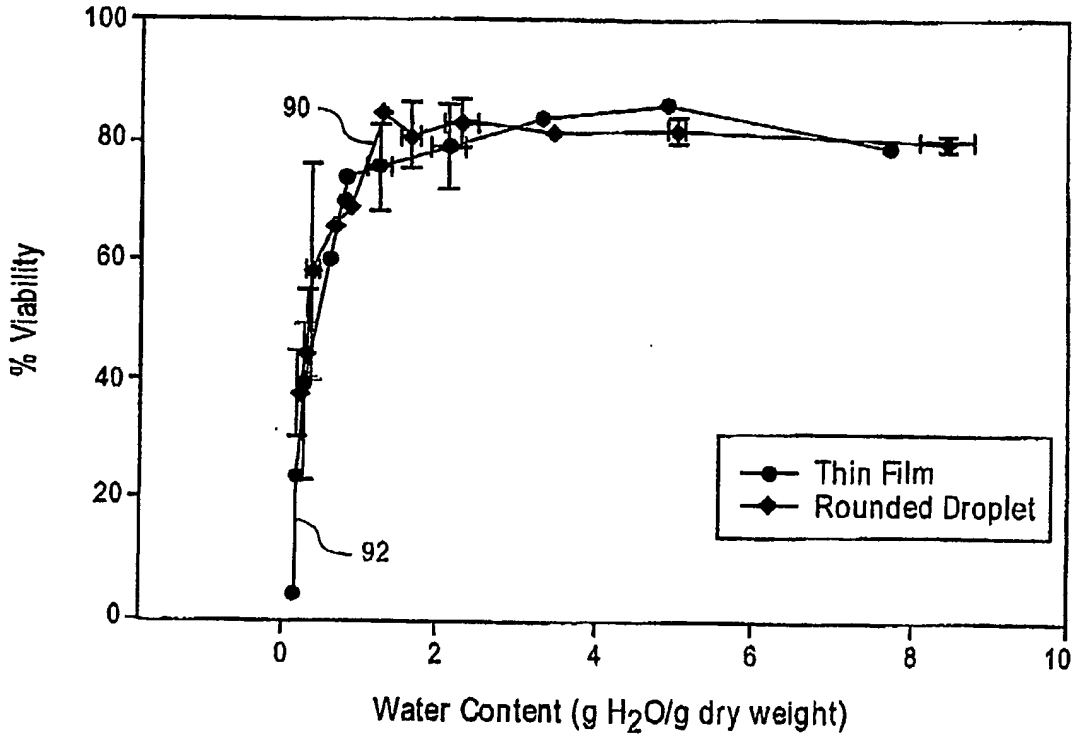

100
80
60
40
20
0
% Viability
90
92
0
2
4
6
8
10
Water Content (g H_2O/g dry weight)
Thin Film
Rounded Droplet

FIG. 7

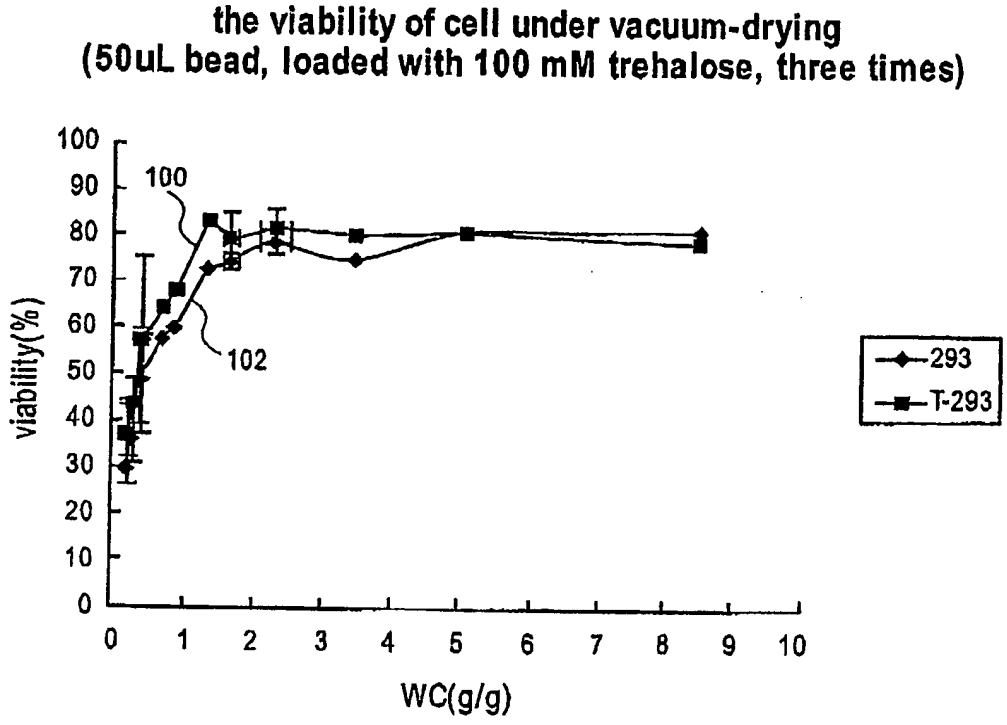

the viability of cell under vacuum-drying
(50uL bead, loaded with 100 mM trehalose, three times)
viability(%)
WC(g/g)
100
102
293
T-293

FIG. 8

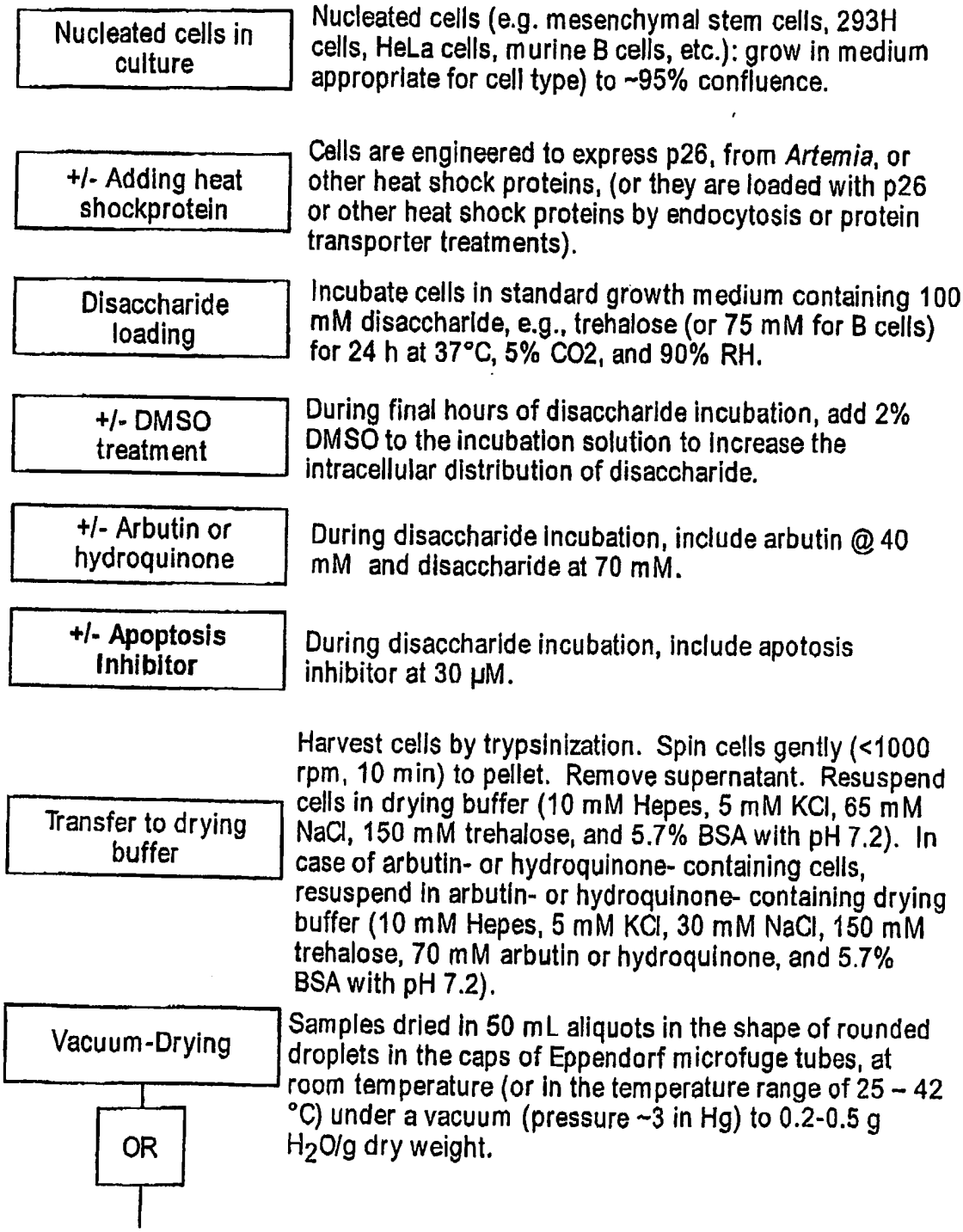
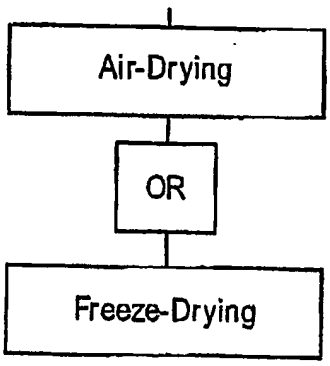
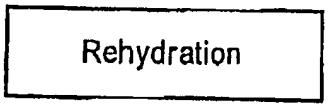

Nucleated cells in culture
Nucleated cells (e.g. mesenchymal stem cells, 293H cells, HeLa cells, murine B cells, etc.): grow in medium appropriate for cell type) to ~95% confluence.
+/- Adding heat shockprotein
Cells are engineered to express p26, from *Artemia*, or other heat shock proteins, (or they are loaded with p26 or other heat shock proteins by endocytosis or protein transporter treatments).
Disaccharide loading
Incubate cells in standard growth medium containing 100 mM disaccharide, e.g., trehalose (or 75 mM for B cells) for 24 h at 37°C, 5% CO2, and 90% RH.
+/- DMSO treatment
During final hours of disaccharide incubation, add 2% DMSO to the incubation solution to increase the intracellular distribution of disaccharide.
+/- Arbutin or hydroquinone
During disaccharide incubation, include arbutin @ 40 mM and disaccharide at 70 mM.
+/- Apoptosis Inhibitor
During disaccharide incubation, include apotosis inhibitor at 30 µM.
Transfer to drying buffer
Harvest cells by trypsinization. Spin cells gently (<1000 rpm, 10 min) to pellet. Remove supernatant. Resuspend cells in drying buffer (10 mM Hepes, 5 mM KCl, 65 mM NaCl, 150 mM trehalose, and 5.7% BSA with pH 7.2). In case of arbutin- or hydroquinone- containing cells, resuspend in arbutin- or hydroquinone- containing drying buffer (10 mM Hepes, 5 mM KCl, 30 mM NaCl, 150 mM trehalose, 70 mM arbutin or hydroquinone, and 5.7% BSA with pH 7.2).
Vacuum-Drying
OR
Samples dried in 50 mL aliquots in the shape of rounded droplets in the caps of Eppendorf microfuge tubes, at room temperature (or in the temperature range of 25 – 42 °C) under a vacuum (pressure ~3 in Hg) to 0.2-0.5 g H_2O/g dry weight.

FIG. 9A



Samples dried in 50 µL aliquots in the shape of rounded droplets in the caps of Eppendorf microfuge tubes (or in 0.5 mL aliquots in sterile Petri dishes), at room temperature under a diffuse stream of dry air to 0.2-0.5 g H_2O/g dry weight.

Cell samples dried in a 10-droplet array (where each drop contains a volume of 50 µL) within BioDRI Flasks on a Lyostar shelf style lyophilizer.



Rehydrate the samples in excess growth medium (+/- arbutin; +/- apoptosis inhibitor).
Replate the cells (on an adhesive surface, such as collagen, fibronectin, or vinculin), and grow in culture at 37 °C, 5% CO_2, and 90% RH. Use the cells for clinical or in vitro applications, as desired.

FIG. 9B